REMARKS

Claims 101-132 are pending in this application. Applicants have amended claims 102 and 110, and added new claims 133-144 without introducing new matter. Claims 101- 144 are now pending.

Preliminary Amendment and Sequence Listing

Applicants note with appreciation the indication that the response filed 01/23/2003 has been entered. Applicants further note that the 06/06/2001 Preliminary Amendment that was filed along with the original application has been entered in part, because of apparently unclear instructions with regard to the sequence listing. Originally, pages 62-177 of the specification contained the sequence listing. It was the intent of the Preliminary Amendment to delete that sequence listing in its entirety in favor of the sequence listing of pages 1-87 accompanying the Preliminary Amendment. Apparently these were treated as part of the original application. For clarity, Applicants hereby replace all previous sequence listings in favor of the sequence listing provided herewith. This sequence listing also includes notation of priority document 60/157,293, which was inadvertently omitted from the version filed with the Preliminary Amendment, but present in the original version. Similarly, inventors Martin GORE and Carol WHITE appear in the original but were omitted from the revised version. These inventors' names have been reintroduced into the bibliographic information on the sequence listing.

Entry of the amendment incorporating this sequence listing is respectfully requested.

Election/Restriction

Applicants note with the appreciation the indication that the election of Group XXVII, claims 93, 95, and 96 has been acknowledged. Applicants respectfully point out an apparent typographical error in paragraph 2 of the Office Action which indicates that new claims 101-132 are drawn to "the elected invention of Group XXVIII" rather than Group XXVII.

Sequence Rules Compliance

The application is objected to for allegedly failing to comply with the sequence rules of 37 C.F.R. §§ 1.821-1.925. In accordance with the Examiner's helpful suggestions, Figure 1 has been replaced with new Figure 1 submitted herewith, which complies with 37 C.F.R.1.821-1.825.

Specification

Applicants note with appreciation the acknowledgement of the claim for priority. The Specification is objected to for not reciting information concerning "priority number information, the relationship of co-pending Application AREN-0050 and 09,364,425 to instant Application and the correct Application number of U.S. Application number of 'AREN-0050'."

Applicants note that the Patent Application Transmittal Letter accompanying the original filing contained, on page 3, an amendment to the first paragraph of the Specification. This amendment included the missing information now requested with the exception of the relationship to AREN-0050 and 09/364,425. That amendment also inadvertently deleted reference to U.S. Provisional 60/156,633 which can be found in the original first paragraph and executed Declaration.

The specification has been amended herein, based on the amendment found on page 3 of the Patent Application Transmittal Letter. The amended text includes paragraph numbering, because although it does not appear in the original specification, the pre-grant publication includes paragraph numbering. Applicants note that although neither the Filing Receipt nor the bibliographical information of the pre-grant publication contain reference to the '633 provisional, apparently in recognition of the amendment in the Transmittal letter, the first paragraph of the publication does not reflect the amendment and therefore includes the reference. Regardless, the first paragraph, as currently amended, now contains all missing serial numbers for priority applications and sets forth information regarding the 60/156,633 application. The text has been amended to eliminate language apparently interpreted by the Office action as implying that priority is claimed to copending applications 09/417,044 (AREN-0050) and 09/364,425. Applicants respectfully assert that the applications are "related" in the sense that they are incorporated by reference into the present

application. A Request for Corrected Filing receipt reflecting the correct priorities accompanies this Response.

The Office Action has questioned the claim for priority made in this application, and has asked Applicants to specify which of the priority documents the present claims relied upon for priority. In response, Applicants respectfully point out that Applicants have previously provided this information in their response filed on January 23, 2003, in which Applicants stated:

> The claims have been amended to comport with the restriction requirement, and for purposes of clarity. New claims 101-132 find support in claims 93, 95 and 96 as filed, in the present specification at pages 30-31, 35, 52-56, in the sequence listing at pages 71-74, and in priority application 60/123,945 (which is incorporated by reference into the present specification - see the present specification at page 1, lines 11-12 and page 2, lines 18-19) at, for example, pages 7, 16-17, 19-20, pages 22-25, and in Figure 1. ...

In their January 23, 2003 response, Applicants also provided a copy of the 60/123,495 provisional application. Accordingly, Applicants assert that the claims of the present application should be afforded the benefit of the March 12, 1999 filing date of the 60/123,945 application. Accordingly, the invention has been fully enabled and in Applicants' possession since at least March 12, 1999. Applicants respectfully request confirmation of the same.

Claim Rejection, 35 USC § 112

Claims 102-108, 110-116 and 119-132 stand rejected under 35 USC § 112, second paragraph as indefinite for allegedly failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically, claims 102, 110, and 119 are rejected for their use of the term "consisting essentially of" in connection with a nucleotide sequence. The remaining claims are rejected as dependent from one of these claims.

While Applicants assert that those of skill in the art would encounter no difficulty making and using the invention according to the claims as written, claims 102 and 110 have been amended merely to recite "consisting of" which should overcome the Examiner's concern. Accordingly, the rejection of claims 102-116 is now moot.

With respect to claim 119, and claims 120-132 dependent therefrom, these claims do not include the "consisting essentially of" language recited in the Office Action. Accordingly, the rejection of claims 119-132 should be withdrawn.

Applicants respectfully submit that all pending claims satisfy the requirements of 35 USC § 112, second paragraph and respectfully request withdrawal of the rejections based thereon.

Claim Rejections – 35 USC § 101 And 35 USC § 112, 1st Paragraph

Claims 101-132 stand rejected under 35 USC § 101 for allegedly lacking support by either a specific and substantial asserted utility or a well-established utility. Applicants note by way of clarification that this section of the Office Action repeatedly refers to "GPR38(V279K)" rather than "GPR38(V297K)", which is the subject of the invention. Throughout these Remarks, where the Office Action is quoted, the correct reference is used without further notation, to avoid confusion.

The reasoning behind the utility rejection fails to acknowledge and incorporate the teachings of priority documents, U.S. Patent No. 6,555,339 and provisional 60/123,945 each of which were incorporated into the specification by reference. Each of those documents teach that when an endogenous GPCR, such as GPR38, is modified according to the teachings of '339, the resultant mutant, such as GP38(V297K), is a non-endogenous constitutively activated version of the original. Thus, the functionality of the non-endogenous constitutively activated version (GPR38(V297K)) is shared with that of the endogenous receptor (GPR38). In fact, the '339 patent recognizes that the mutated version has high pharmacological fidelity to the original.

Thus, although the statements on page 7 of the Office Action discussing the highly divergent nature and effects of various GPCRs may normally be relevant, here, where the mutated receptor, GPR38(V297K), is designed and used specifically because of its shared characteristics and pharmacological fidelity to its parent, GPR38, the general caveat in the art is overcome. Accordingly, Applicants respectfully assert that those skilled in the art, reading Applicants' specification and priority documents, would readily and immediately equate the functionalities of GPR38 and GPR38(V297K).

Thus, although the Action repeatedly alleges no well-established utility or asserted specific and substantial utility is present, Applicants respectfully assert that well-established and asserted utilities exist for GPR38 and, thus, for GPR38(V297K).

Initially, Applicants note that either a well-established utility or an asserted specific and substantial utility is sufficient to satisfy 35 USC § 101. MPEP § 2107.02(b) indicates that even in the absence of statements asserting a specific and substantial utility, "if an invention has a well-established utility, rejections under 35 USC 101 and 35 USC 112, first paragraph, based on lack of utility should not be imposed. *In re Folkers*, 344 F.2d 970, 145 USPQ 390 (CCPA 1965).".

GPR 38 has a Well-Established Utility

GPR38 has a well-established utility for the prevention of exacerbation of or treatment of Graves' disease. As indicated on pages 8 and 18 of provisional application no. 60/123,945, it was known at the time of filing that GPR38, which was disclosed as a GPCR, was expressed in the thyroid [McKee et al., Genomics (1997) 46:426-34]. Applicants have shown that activation of GPR38 leads to an increase in intracellular cAMP (Figure 1 of 60/123,945).

Furthermore, it was appreciated at the time of filing that the most common hyperthyroid syndrome, Graves' disease, was an autoimmune disease characterized by IgG antibodies that bound to and activated the TSH receptor [Goodman & Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition (1996), McGraw-Hill, left column of page 1394; filed previously by IDS]. It was also appreciated at the time of filing that the TSH receptor is a GPCR whose activation leads to an increase in intracellular cAMP [Goodman & Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition (1996), McGraw-Hill, right column of page 1390; filed previously by IDS]. It therefore follows that at the time of filing, an agent that inhibited a thyroid pathway leading to an increase in intracellular cAMP would have well-established utility for the prevention of exacerbation of or treatment of Graves' disease. An inverse agonist of GPR38 is such an agent. Thus, GPR38 (V297K), by virtue of its high pharmacological fidelity to GPR38 and its expanded screening window for an inverse agonist relative to GPR38 (e.g., ~9500 LCPS units versus ~0 LCPS units; Figure 1 of 60/123,945), has a well-established utility for the prevention of exacerbation of

or treatment of Graves' disease. Those skilled in the art would recognize this well-established utility.

Specific and Substantial Utility

Applicants note with appreciation the indication on page 6 that "Applicant has asserted utilities for the specifically claimed invention." MPEP § 2107.02(b) indicates that asserted utilities should be presumed as valid absent evidence to doubt the truth of the assertion. Applicants respectfully submit that no such evidence is present on this record.

The Action reiterates the basic rejection and underlying reasoning several times. Perhaps, it is best summarized on page 6, line18 through page 7, line 1, which alleges:

> No disclosure is provided within the instant specification on what specific function a putative GPR38(V297K), protein possesses, or ligands that bind, promoters that activate; nor are any cell types/tissues disclosed that specifically express this protein; nor are any disease states disclosed that are directly related to GPR38(V297K).

As discussed above, GPR38(V297K) has high pharmacological fidelity to its parent, GPR38. Since GPR38(V297K) results from a planned mutation, it is not naturally expressed in any cell type or tissue. However, GPR38 is, and was known to be, expressed in the thyroid, which is known to be involved in Graves' disease as discussed above. The Action has provided no evidence that would lead those skilled in the art to question this asserted utility. Accordingly, the rejection should be withdrawn.

The Action continues, noting in several places (e.g. page 7, lines 9-12) that

> The utility of GPR38(V297K), cannot be implicated solely from homology to known G-protein coupled receptors because the art does not provide teaching stating that all members of a sub-family of G-protein coupled receptors must have the same effects, the same ligands and be involved in the same disease states, the art discloses evidence to the contrary.

GPR38(V297K) and GPR38 differ by a single amino acid. This single amino acid is not chosen at random, but rather by a specific algorithm set forth in the '339 patent. In this instance, the substitution takes place at codon 297 (thus, V297K). The '339 patent and '945 provisional application clearly establish that it is mutation at this location in GPR38 that yields the constitutively

active version of the endogenous receptor and not some other receptor. Thus, mere homology does not enter into the equation. The utility based on the underlying endogenous receptor, GPR38, is based, not on homology, but upon the specific application of the teachings of the '339 patent and the '945 provisional application concerning modification of GPR38 at codon 297. Accordingly, reliance on the known properties of GPR38 is not misplaced as being "implicated solely from homology."

At page 6, lines 15-18 the Office Action questions the status of GPR38(V297K) as a GPCR, indicating that Applicants specification discloses that GPR38(V297K) "may" be a GPCR leaving those skilled in the art to "speculate" as to whether it is or not. This is not only in direct contradiction to Applicants' specification, which clearly states throughout the specification (e.g. Table G indicating GPR38(V297K) as a non-endogenous human GPCR) that GPR38(V297K) like its parent, GPR38, is a GPCR, but also is in direct conflict with the statement of page 7 of the Office Action stating:

> The GPR38(V297K), of instant invention is considered by the examiner to be a member of the orphan receptor of G-protein coupled receptors i.e. seven transmembrane receptor with no known endogenous ligands.

Thus, both Applicants and the Examiner appear to agree that GPR38(V297K) is in fact a GPCR.

Accordingly, Applicants respectfully assert that GPR38(V297K) has a specific and substantial utility in that inverse antagonism of GPR38(V297K), and by implication GPR38, is useful in preventing exacerbation of or treating Graves' disease.

In light of the foregoing discussion illuminating either or both of a well-established utility or at least one specific and substantial utility, Applicants respectfully assert that the requirements of 35 USC § 101 have been met. Withdrawal of the rejections based thereon is respectfully requested.

Rejection Under 35 USC § 112, First Paragraph

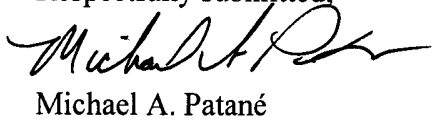
Claims 101-132 stand rejected under 35 USC § 112, first paragraph, for alleged lack of enablement since the claims allegedly lack utility. In light of the arguments above, Applicants respectfully submit that those skilled in the art would recognize both the utility of the invention and how to use it. Applicants respectfully request withdrawal of the 35 USC § 112 rejection.

Information Disclosure Statement

In a handwritten note on its last page, the Office Action states that the references cited in the IDS filed on January 23, 2003 were unavailable at the time of Examination. The IDS was filed contemporaneously with the Response to Restriction Requirement and the claims under current examination. Accordingly, both the references and claims should have been available at the same time. Applicants respectfully request that any future action relying on any reference cited in the January 23, 2003 IDS be non-final.

Applicants assert that the claims are in condition for allowance, and respectfully request notification to that effect. Should the Office have any questions, Applicants invite the Office to contact the undersigned at (215) 665-2000 to discuss any issues unresolved by this Amendment. A Notice of Allowance is earnestly solicited.

Respectfully submitted,



Date: September 24, 2003

Michael A. Patané
Reg. No. 42, 982

COZEN O'CONNOR, P.C.
1900 Market Street
Philadelphia, PA 19103-3508
Telephone: 215.665.6966
Facsimile: 215.665.2013

Express Mail Label No. EL247533440US

IN THE UNITED STATES PAPTENT AND TRADEMARK OFFICE

PROVISIONAL PATENT APPLICATION OF:

Chen W. LIAW, Derek T. CHALMERS and Dominic P. BEHAN

FOR:

NON-ENDOGENOUS, CONSTITUTIVELY ACTIVATED HUMAN G PROTEIN-COUPLED ORPHAN RECEPTOR: GPR38

ARENA PHARMACEUTICALS, INC.
6166 Nancy Ridge Drive
San Diego, CA 92121

NON-ENDOGENOUS CONSTITUTIVELY ACTIVATED
HUMAN G PROTEIN-COUPLED ORPHAN RECEPTOR: GPR38

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NON-ENDOGENOUS CONSTITUTIVELY ACTIVATED HUMAN G PROTEIN-COUPLED ORPHAN RECEPTOR: GPR38

The benefit of commonly owned U.S. Serial Number 09/170,496, filed via Express Mail on October 13, 1998, is hereby claimed.

FIELD OF THE INVENTION

The invention disclosed in this patent document relates to transmembrane receptors, more particularly to G protein-coupled receptors for which the endogenous ligand is unknown ("orphan GPCR receptors"), and most particularly, to a mutated (non-endogenous) version of an orphan GPCR receptor, GPR38, that by virtue of the mutation is constitutively active.

BACKGROUND OF THE INVENTION

Although a number of receptor classes exist in humans, by far the most abundant and therapeutically relevant is represented by the G protein-coupled receptor (GPCR or GPCRs) class. It is estimated that there are some 100,000 genes within the human genome, and of these, approximately 2% or 2,000 genes, are estimated to code for GPCRs. Of these, there are approximately 100 GPCRs for which the endogenous ligand that binds to the GPCR has been identified. Because of the significant time-lag that exists between the discovery of an endogenous GPCR and its endogenous ligand, it can be presumed that the remaining 1,900 GPCRs will be identified and characterized long before the endogenous ligands for these receptors are identified. Indeed, the rapidity by which the Human Genome Project is sequencing the 100,000 human genes indicates that the remaining human GPCRs

will be fully sequenced within the next few years. Nevertheless, and despite the efforts to sequence the human genome, it is still very unclear as to how scientists will be able to rapidly, effectively and efficiently exploit this information to improve and enhance the human condition. The present invention is geared towards this important objective.

Receptors, including GPCRs, for which the endogenous ligand has been identified are referred to as "known" receptors, while receptors for which the endogenous ligand has not been identified are referred to as "orphan" receptors. This distinction is not merely semantic, particularly in the case of GPCRs. GPCRs represent an important area for the development of pharmaceutical products: from approximately 20 of the 100 known GPCRs, 60% of all prescription pharmaceuticals have been developed. Thus, the orphan GPCRs are to the pharmaceutical industry what gold was to California in the late 19th century – an opportunity to drive growth, expansion, enhancement and development. A serious drawback exists, however, with orphan receptors relative to the discovery of novel therapeutics. This is because the traditional approach to the discovery and development of pharmaceuticals has required access to both the receptor *and* its endogenous ligand. Thus, heretofore, orphan GPCRs have presented the art with a tantalizing and undeveloped resource for the discovery of pharmaceuticals.

Under the traditional approach to the discovery of potential therapeutics, it is generally the case that the receptor is first identified. Before drug discovery efforts can be initiated, elaborate, time consuming and expensive procedures are typically put into place in order to identify, isolate and generate the receptor's endogenous ligand – this process can require from between three and ten years per receptor, at a cost of about $5million (U.S.) per receptor. These time and financial resources must be expended before the traditional

approach to drug discovery can commence. This is because traditional drug discovery techniques rely upon so-called "competitive binding assays" whereby putative therapeutic agents are "screened" against the receptor in an effort to discover compounds that either block the endogenous ligand from binding to the receptor ("antagonists"), or enhance or mimic the effects of the ligand binding to the receptor ("agonists"). The overall objective is to identify compounds that prevent cellular activation when the ligand binds to the receptor (the antagonists), or that enhance or increase cellular activity that would otherwise occur if the ligand was properly binding with the receptor (the agonists). Because the endogenous ligands for orphan GPCRs are by definition not identified, the ability to discover novel and unique therapeutics to these receptors using traditional drug discovery techniques is not possible. The present invention, as will be set forth in greater detail below, overcomes these and other severe limitations created by such traditional drug discovery techniques.

GPCRs share a common structural motif. All these receptors have seven sequences of between 22 to 24 hydrophobic amino acids that form seven alpha helices, each of which spans the membrane (each span is identified by number, *i.e.*, transmembrane-1 (TM-1), transmebrane-2 (TM-2), etc.). The transmembrane helices are joined by strands of amino acids between transmembrane-2 and transmembrane-3, transmembrane-4 and transmembrane-5, and transmembrane-6 and transmembrane-7 on the exterior, or "extracellular" side, of the cell membrane (these are referred to as "extracellular" regions 1, 2 and 3 (EC-1, EC-2 and EC-3), respectively). The transmembrane helices are also joined by strands of amino acids between transmembrane-1 and transmembrane-2, transmembrane-3 and transmembrane-4, and transmembrane-5 and transmembrane-6 on the interior, or "intracellular" side, of the cell membrane (these are referred to as "intracellular" regions 1, 2

and 3 (IC-1, IC-2 and IC-3), respectively). The "carboxy" ("C") terminus of the receptor lies in the intracellular space within the cell, and the "amino" ("N") terminus of the receptor lies in the extracellular space outside of the cell.

Generally, when an endogenous ligand binds with the receptor (often referred to as "activation" of the receptor), there is a change in the conformation of the intracellular region that allows for coupling between the intracellular region and an intracellular "G-protein." It has been reported that GPCRs are "promiscuous" with respect to G proteins, *i.e.*, that a GPCR can interact with more than one G protein. *See*, Kenakin, T., 43 *Life Sciences* 1095 (1988). Although other G proteins exist, currently, Gq, Gs, Gi, and Go are G proteins that have been identified. Endogenous ligand-activated GPCR coupling with the G-protein begins a signaling cascade process (referred to as "signal transduction"). Under normal conditions, signal transduction ultimately results in cellular activation or cellular inhibition. It is thought that the IC-3 loop as well as the carboxy terminus of the receptor interact with the G protein. A principal focus of this invention is directed to the transmembrane-6 (TM6) region and the intracellular-3 (IC3) region of the GPCR.

Under physiological conditions, GPCRs exist in the cell membrane in equilibrium between two different conformations: an "inactive" state and an "active" state. A receptor in an inactive state is unable to link to the intracellular signaling transduction pathway to produce a biological response. Changing the receptor conformation to the active state allows linkage to the transduction pathway (via the G-protein) and produces a biological response.

A receptor may be stabilized in an active state by an endogenous ligand or a compound such as a drug. Recent discoveries, including but not exclusively limited to

modifications to the amino acid sequence of the receptor, provide means other than endogenous ligands or drugs to promote and stabilize the receptor in the active state conformation. These means effectively stabilize the receptor in an active state by simulating the effect of an endogenous ligand binding to the receptor. Stabilization by such ligand-independent means is termed "constitutive receptor activation."

As noted above, the use of an orphan receptor for screening purposes has not been possible. This is because the traditional "dogma" regarding screening of compounds mandates that the ligand for the receptor be known. By definition, then, this approach has no applicability with respect to orphan receptors. Thus, by adhering to this dogmatic approach to the discovery of therapeutics, the art, in essence, has taught and has been taught to forsake the use of orphan receptors unless and until the endogenous ligand for the receptor is discovered. Given that there are an estimated 2,000 G protein coupled receptors, the majority of which are orphan receptors, such dogma castigates a creative, unique and distinct approach to the discovery of therapeutics.

The present invention relates to the human GPR38 receptor. GPR38 was cloned and sequenced in 1997. McKee, K.K.. et al, 46 *Genomics.* 426 (1997), *see* Figure 1 of McKee for deduced amino acid sequence. According to McKee, the endogenous ligand for GPR38 is unknown – indeed, as noted by McKee: "Gaining an understanding of the normal physiological role of [GPR38] will initially involve...identification of [its] endogenous ligand(s)." Thus, GPR38 is an orphan GPCR. *See also*, GenBank Accession Number AF034632 for submitted amino acid and nucleic acid sequences. GPR38 has been reported to be closely related to the type 1 neurotensin receptor-1 and growth hormone

secretarogue receptor of the GPCR, and is reportedly expressed in thyroid gland, stomach and bone marrow (*see*, McKee *supra*).

As will be set forth and disclosed in greater detail below, utilization of a mutational cassette to modify the endogenous sequence of human GPR38 leads to a constitutively activated version of that receptor. This non-endogenous, constitutively activated version of human GPR38 can be utilized, *inter alia*, for the screening of candidate compounds to directly identify compounds of, *e.g.*, therapeutic relevance.

SUMMARY OF THE INVENTION

Disclosed herein is a mutated version of human GPR38, which mutation leads to constitutive activation of this receptor.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides graphic results of comparative analysis of endogenous GPR38 ("WT") versus non-endogenous, constitutively activated GPR38 ("V297K") (control is designated "CMV").

DETAILED DESCRIPTION

The scientific literature that has evolved around receptors has adopted a number of terms to refer to ligands having various effects on receptors. For clarity and consistency, the following definitions will be used throughout this patent document. To the extent that these definitions conflict with other definitions for these terms, the following definitions shall control:

AGONISTS shall mean compounds that activate the intracellular response when they bind to the receptor, or enhance GTP binding to membranes.

AMINO ACID ABBREVIATIONS used herein are set below:

ALANINE	ALA	A
ARGININE	ARG	R
ASPARAGINE	ASN	N
ASPARTIC ACID	ASP	D
CYSTEINE	CYS	C
GLUTAMIC ACID	GLU	E
GLUTAMINE	GLN	Q
GLYCINE	GLY	G
HISTIDINE	HIS	H
ISOLEUCINE	ILE	I
LEUCINE	LEU	L
LYSINE	LYS	K
METHIONINE	MET	M
PHENYLALANINE	PHE	F
PROLINE	PRO	P
SERINE	SER	S
THREONINE	THR	T
TRYPTOPHAN	TRP	W
TYROSINE	TYR	Y
VALINE	VAL	V

PARTIAL AGONISTS shall mean compounds which activate the intracellular response when they bind to the receptor to a lesser degree/extent than do agonists, or enhance GTP binding to membranes to a lesser degree/extent than do agonists

ANTAGONIST shall mean compounds that competitively bind to the receptor at the same site as the agonists but which do not activate the intracellular response initiated by

the active form of the receptor, and can thereby inhibit the intracellular responses by agonists or partial agonists. ANTAGONISTS do not diminish the baseline intracellular response in the absence of an agonist or partial agonist.

CANDIDATE COMPOUND shall mean a molecule (for example, and not limitation, a chemical compound) which is amenable to a screening technique. Preferably, the phrase "candidate compound" does not include compounds which were publicly known to be compounds selected from the group consisting of inverse agonist, agonist or antagonist to a receptor, as previously determined by an indirect identification process ("indirectly identified compound"); more preferably, not including an indirectly identified compound which has previously been determined to have therapeutic efficacy in at least one mammal; and, most preferably, not including an indirectly identified compound which has previously been determined to have therapeutic utility in humans.

CODON shall mean a grouping of three nucleotides (or equivalents to nucleotides) which generally comprise a nucleoside (adenosine (A), guanosine (G), cytidine (C), uridine (U) and thymidine (T)) coupled to a phosphate group and which, when translated, encodes an amino acid.

COMPOUND EFFICACY shall mean a measurement of the ability of a compound to inhibit or stimulate receptor functionality, as opposed to receptor binding affinity. A preferred means of detecting compound efficacy is via measurement of, *e.g.*, [^{35}S]GTPγS binding, as further disclosed in the Example section of this patent document.

CONSTITUTIVELY ACTIVATED RECEPTOR shall mean a receptor subject to constitutive receptor activation. In accordance with the invention disclosed herein, a non-

endogenous, human constitutively activated GPR38 is one that has been sequence mutated relative to its endogenous sequence.

CONSTITUTIVE RECEPTOR ACTIVATION shall mean stabilization of a receptor in the active state by means other than binding of the receptor with its endogenous ligand or a chemical equivalent thereof. Preferably, a G protein-coupled receptor subjected to constitutive receptor activation in accordance with the invention disclosed herein evidences at least a 10% difference in response (increase or decrease, as the case may be) to the signal measured for constitutive activation as compared with the endogenous form of that GPCR, more preferably, about a 25% difference in such comparative response, and most preferably about a 50% difference in such comparative response. When used for the purposes of directly identifying candidate compounds, it is most preferred that the signal difference be at least about 50% such that there is a sufficient difference between the endogenous signal and the non-endogenous signal to differentiate between selected candidate compounds. In most instances, the "difference" will be an increase in signal; however, with respect to Gs-coupled GPCRs, the "difference" measured is preferably a decrease, as will be set forth in greater detail below.

CONTACT or **CONTACTING** shall mean bringing at least two moieties together, whether in an in vitro system or an in vivo system.

DIRECTLY IDENTIFYING or **DIRECTLY IDENTIFIED**, in relationship to the phrase "candidate compound", shall mean the screening of a candidate compound against a constitutively activated G protein-coupled receptor, and assessing the compound efficacy of such compound. This phrase is, under no circumstances, to be interpreted or

understood to be encompassed by or to encompass the phrase "indirectly identifying" or "indirectly identified."

ENDOGENOUS shall mean a material that is naturally produced by the genome of the species. ENDOGENOUS in reference to, for example and not limitation, GPCR, shall mean that which is naturally produced by a human, an insect, a plant, a bacterium, or a virus. By contrast, the term **NON-ENDOGENOUS** in this context shall mean that which is not naturally produced by the genome of a species. For example, and not limitation, a receptor which is not constitutively active in its endogenous form, but when mutated by using the cassettes disclosed herein and thereafter becomes constitutively active, is most preferably referred to herein as a "non-endogenous, constitutively activated receptor." Both terms can be utilized to describe both "in vivo" and "in vitro" systems. For example, and not limitation, in a screening approach, the endogenous or non-endogenous receptor may be in reference to an in vitro screening system whereby the receptor is expressed on the cell-surface of a mammalian cell. As a further example and not limitation, where the genome of a mammal has been manipulated to include a non-endogenous constitutively activated receptor, screening of a candidate compound by means of an in vivo system is viable.

HOST CELL shall mean a cell capable of having a Plasmid and/or Vector incorporated therein. In the case of a prokaryotic Host Cell, a Plasmid is typically replicated as an autonomous molecule as the Host Cell replicates (generally, the Plasmid is thereafter isolated for introduction into a eukaryotic Host Cell); in the case of a eukaryotic Host Cell, a Plasmid is integrated into the cellular DNA of the Host Cell such that when the eukaryotic Host Cell replicates, the Plasmid replicates. Preferably, for the purposes of the invention

disclosed herein, the Host Cell is eukaryotic, more preferably, mammalian, and most preferably selected from the group consisting of 293, 293T and COS-7 cells.

INDIRECTLY IDENTIFYING or **INDIRECTLY IDENTIFIED** means the traditional approach to the drug discovery process involving identification of an endogenous ligand specific for an endogenous receptor, screening of candidate compounds against the receptor for determination of those which interfere and/or compete with the ligand-receptor interaction, and assessing the efficacy of the compound for affecting at least one second messenger pathway associated with the activated receptor.

INHIBIT or **INHIBITING,** in relationship to the term "response" shall mean that a response is decreased or prevented in the presence of a compound as opposed to in the absence of the compound.

INVERSE AGONISTS shall mean compounds which bind to either the endogenous form of the receptor or to the constitutively activated form of the receptor, and which inhibit the baseline intracellular response initiated by the active form of the receptor below the normal base level of activity which is observed in the absence of agonists or partial agonists, or decrease GTP binding to membranes. Preferably, the baseline intracellular response is inhibited in the presence of the inverse agonist by at least 30%, more preferably by at least 50%, and most preferably by at least 75%, as compared with the baseline response in the absence of the inverse agonist.

KNOWN RECEPTOR shall mean an endogenous receptor for which the endogenous ligand specific for that receptor has been identified.

LIGAND shall mean an endogenous, naturally occurring molecule specific for an endogenous, naturally occurring receptor.

MUTANT or **MUTATION** in reference to an endogenous receptor's nucleic acid and/or amino acid sequence shall mean a specified change or changes to such endogenous sequences such that a mutated form of an endogenous, non-constitutively activated receptor evidences constitutive activation of the receptor. In terms of equivalents to specific sequences, a subsequent mutated form of a human receptor is considered to be equivalent to a first mutation of the human receptor if (a) the level of constitutive activation of the subsequent mutated form of the receptor is substantially the same as that evidenced by the first mutation of the receptor; and (b) the percent sequence (amino acid and/or nucleic acid) homology between the subsequent mutated form of the receptor and the first mutation of the receptor is at least about 80%, more preferably at least about 90% and most preferably at least 95%. Ideally, and owing to the fact that the most preferred mutation disclosed herein for achieving constitutive activation includes a single amino acid and/or codon change between the endogenous and the non-endogenous forms of the GPCR, the percent sequence homology should be at least 98%.

ORPHAN RECEPTOR shall mean an endogenous receptor for which the endogenous ligand specific for that receptor has not been identified or is not known.

PHARMACEUTICAL COMPOSITION shall mean a composition comprising at least one active ingredient, whereby the composition is amenable to investigation for a specified, efficacious outcome in a mammal (for example, and not limitation, a human). Those of ordinary skill in the art will understand and appreciate the techniques appropriate for determining whether an active ingredient has a desired efficacious outcome based upon the needs of the artisan.

PLASMID shall mean the combination of a Vector and cDNA. Generally, a Plasmid is introduced into a Host Cell for the purpose of replication and/or expression of the cDNA as a protein.

STIMULATE or **STIMULATING**, in relationship to the term "response" shall mean that a response is increased in the presence of a compound as opposed to in the absence of the compound.

TRANSVERSE or **TRANSVERSING**, in reference to either a defined nucleic acid sequence or a defined amino acid sequence, shall mean that the sequence is located within at least two different and defined regions. For example, in an amino acid sequence that is 10 amino acid moieties in length, where 3 of the 10 moieties are in the TM6 region of a GPCR and the remaining 7 moieties are in the IC3 region of the GPCR, the 10 amino acid moiety can be described as transversing the TM6 and IC3 regions of the GPCR.

VECTOR in reference to cDNA shall mean a circular DNA capable of incorporating at least one cDNA and capable of incorporation into a Host Cell.

The order of the following sections is set forth for presentational efficiency and is not intended, nor should be construed, as a limitation on the disclosure or the claims to follow.

A. Introduction

The traditional study of receptors has always proceeded from the a priori assumption (historically based) that the endogenous ligand must first be identified before discovery could proceed to find antagonists and other molecules that could affect the receptor. Even in cases where an antagonist might have been known first, the search immediately extended to

looking for the endogenous ligand. This mode of thinking has persisted in receptor research even after the discovery of constitutively activated receptors. What has not been heretofore recognized is that it is the active state of the receptor that is most useful for discovering agonists, partial agonists, and inverse agonists of the receptor. For those diseases which result from an overly active receptor or an under-active receptor, what is desired in a therapeutic drug is a compound which acts to diminish the active state of a receptor or enhance the activity of the receptor, respectively, not necessarily a drug which is an antagonist to the endogenous ligand. This is because a compound that reduces or enhances the activity of the active receptor state need not bind at the same site as the endogenous ligand. Thus, as taught by a method of this invention, any search for therapeutic compounds should start by screening compounds against the ligand-independent active state.

Screening candidate compounds against non-endogenous, constitutively activated GPR38 allows for the direct identification of candidate compounds which act at this cell surface receptor, without requiring any prior knowledge or use of the receptor's endogenous ligand. By assessing areas within the body where the endogenous version of human GPR38 is expressed and/or over-expressed, it is possible to determine related disease/disorder states which are associated with the expression and/or over-expression of the receptor; such an approach is disclosed in this patent document.

In its endogenous form, GPR38 is not constitutively active, *i.e.*, GPR38 signaling via G protein is ligand-dependent. Thus, it is not feasible to search directly for, *e.g.*, inverse agonists, to endogenous GPR38. However, by the mutation approach disclosed in this patent document, GPR38 can be constitutively activated such that screening of candidate compounds against the non-endogenous, constitutively activated GPR38 orphan receptor

allows for the direct identification of candidate compounds as *e.g.*, inverse agonists. In the present invention, endogenous GPR38 was mutated, such that the valine residue at codon 297 was changed to a lysine residue, resulting in a non-endogenous, constitutively activate GPR38. Although the non-endogenous amino acid at this position can be any of the amino acids (other than the endogenous valine residue), most preferably, the non-endogenous amino acid is lysine.

Because there are only 20 naturally occurring amino acids (although the use of non-naturally occurring amino acids is also viable), selection of a particular non-endogenous amino acid for substitution at codon 297 is viable and allows for efficient selection of a non-endogenous amino acid that fits the needs of the investigator. However, as noted, the more preferred non-endogenous amino acids at codon 297 are lysine, hisitidine, arginine and alanine, with lysine being most preferred. Those of ordinary skill in the art are credited with the ability to readily determine proficient methods for changing the sequence of a codon to achieve a desired mutation.

B. Disease/Disorder Identification and/or Selection

As will be set forth in greater detail below, most preferably inverse agonists to the non-endogenous, constitutively activated GPR38 receptor can be identified by the methodologies of this invention. Such inverse agonists are ideal candidates as lead compounds in drug discovery programs for treating diseases related to this receptor. Because of the ability to directly identify inverse agonists to the GPR38 receptor, thereby allowing for the development of pharmaceutical compositions, a search for diseases and disorders associated with the GPR38 receptor is relevant. For example, scanning both diseased and normal tissue samples for the presence of the GPR38 receptor now becomes

more than an academic exercise or one which might be pursued along the path of identifying an endogenous ligand to GPR38. Tissue scans can be conducted across a broad range of healthy and diseased tissues. Such tissue scans provide a preferred first step in associating a specific receptor with a disease and/or disorder. As noted above, GPR38 is reportedly expressed in thyroid gland, stomach and bone marrow (McKee, *supra*)

Preferably, the DNA sequence of the GPR38 receptor is used to make a probe for (a) dot-blot analysis against tissue-mRNA, and/or (b) RT-PCR identification of the expression of the receptor in tissue samples. The presence of a receptor in a tissue source, or a diseased tissue, or the presence of the receptor at elevated concentrations in diseased tissue compared to a normal tissue, can be preferably utilized to identify a correlation with a treatment regimen, including but not limited to, a disease associated with that disease. Receptors can equally well be localized to regions of organs by this technique. Based on the known functions of the specific tissues to which the receptor is localized, the putative functional role of the receptor can be deduced.

C. Screening of Candidate Compounds

1. Generic GPCR screening assay techniques

When a G protein receptor becomes constitutively active, it binds to a G protein (Gq, Gs, Gi, Go) and stimulates the binding of GTP to the G protein. The G protein then acts as a GTPase and slowly hydrolyzes the GTP to GDP, whereby the receptor, under normal conditions, becomes deactivated. However, constitutively activated receptors continue to exchange GDP to GTP. A non-hydrolyzable analog of GTP, [^{35}S]GTPγS, can be used to monitor enhanced binding to membranes which express constitutively activated receptors. It is reported that [^{35}S]GTPγS can be used to monitor G protein coupling to membranes in the

absence and presence of ligand. An example of this monitoring, among other examples well-known and available to those in the art, was reported by Traynor and Nahorski in 1995. The preferred use of this assay system is for initial screening of candidate compounds because the system is generically applicable to all G protein-coupled receptors regardless of the particular G protein that interacts with the intracellular domain of the receptor.

2. Specific GPCR screening assay techniques

Once candidate compounds are identified using the "generic" G protein-coupled receptor assay (*i.e.* an assay to select compounds that are agonists, partial agonists, or inverse agonists), further screening to confirm that the compounds have interacted at the receptor site is preferred. For example, a compound identified by the "generic" assay may not bind to the receptor, but may instead merely "uncouple" the G protein from the intracellular domain. In the case of GPR38, it has been determined that this receptor couples the G protein Gs. Gs is known to activate the enzyme adenylyl cyclase, which is necessary for catalyzing the conversion of ATP to cAMP. Thus, a non-endogenous, constitutively activated version of human GPR38 would be expected to be associated with increased levels of cAMP. Thus, following direct identification of candidate compounds via a generic assay, an assay which is based upon responses mediated by the G protein associated with the receptor, *i.e.*, a "second messenger" assay, is preferably used to confirm or refine the direct identification results obtained from a generic assay.

Assays that detect cAMP can be utilized to determine if a candidate compound is e.g., an inverse agonist to a Gs-associated receptor (*i.e.*, such a compound would decrease the levels of cAMP) or a Gi-associated receptor (or Go-associated receptor) (*i.e.*, such a candidate compound would increase the levels of cAMP). A variety of approaches

known in the art for measuring cAMP can be utilized; a preferred approach relies upon the use of anti-cAMP antibodies in an ELISA-based format. Another type of assay, and most preferred, utilizes a whole cell second messenger reporter system assay. Promoters on genes drive the expression of the proteins that a particular gene encodes. Cyclic AMP drives gene expression by promoting the binding of a cAMP-responsive DNA binding protein or transcription factor (CREB) which then binds to the promoter at specific sites called cAMP response elements and drives the expression of the gene. Reporter systems can be constructed which have a promoter containing multiple cAMP response elements before the reporter gene, *e.g.*, β-galactosidase or luciferase. Thus, a constitutively activated Gs-linked receptor causes the accumulation of cAMP that then activates the gene and expression of the reporter protein. The reporter protein such as β-galactosidase or luciferase can then be detected using standard biochemical assays (Chen W. *et al.* 226(2) *Anal. Biochem.* 349(1995)). Several reporter plasmids are known and available in the art for measuring a second messenger assay. It is considered well within the skilled artisan to determine an appropriate reporter plasmid for a particular gene expression based primarily upon the particular needs of the artisan.

The foregoing specific assay approach can, of course be utilized to initially directly identify candidate compounds, rather than by using the generic assay approach. Such a selection is primarily a matter of choice of the artisan.

D. Medicinal Chemistry

Generally, but not always, direct identification of candidate compounds is preferably conducted in conjunction with compounds generated via combinatorial chemistry techniques, whereby thousands of compounds are randomly prepared for such

analysis. Generally, the results of such screening will be compounds having unique core structures; thereafter, these compounds are preferably subjected to additional chemical modification around a preferred core structure(s) to further enhance the medicinal properties thereof. Such techniques are known to those in the art and will not be addressed in detail in this patent document.

E. Pharmaceutical compositions

Candidate compounds selected for further development can be formulated into pharmaceutical compositions using techniques well known to those in the art. Suitable pharmaceutically-acceptable carriers are available to those in the art; for example, see Remington's Pharmaceutical Sciences, 16th Edition, 1980, Mack Publishing Co., (Oslo et al., eds.)

F. Other Utility

Although a preferred use of the non-endogenous versions of human GPR38 is for the direct identification of candidate compounds as inverse agonists, agonists or partial agonists (preferably for use as pharmaceutical agents), this version of human GPR38 can also be utilized in research settings. For example, *in vitro* and *in vivo* systems incorporating GPR38 can be utilized to further elucidate and understand the roles GPR38 plays in the human condition, both normal and diseased, as well as understanding the role of constitutive activation as it applies to understanding the signaling cascade. A value in non-endogenous human GPR38 is that its utility as a research tool is enhanced in that, because of its unique features, non-endogenous GPR38 can be used to understand the role of GPR38 in the human body before the endogenous ligand therefor is identified. Other uses of the disclosed

receptors will become apparent to those in the art based upon, *inter alia*, a review of this patent document.

EXAMPLES

The following examples are presented for purposes of elucidation, and not limitation, of the present invention. While specific nucleic acid and amino acid sequences are disclosed herein, those of ordinary skill in the art are credited with the ability to make minor modifications to these sequences while achieving the same or substantially similar results reported below.

Example 1
PREPARATION OF ENDOGENOUS, NON-CONSTITUTIVELY ACTIVATED GPR38

PCR was performed by combining two PCR fragments, using human genomic cDNA as template and rTth polymerase (Perkin Elmer) with the buffer system provided by the manufacturer, 0.25 μM of each primer, and 0.2 mM of each 4 nucleotides. The cycle condition for each PCR reaction was 30 cycles of 94°C for 1 min, 62°C for 1 min and 72°C for 2 min.

The first fragment was amplified with the 5' PCR primer that contained a end site with the following sequence:

5' -ACCATGGGCAGCCCCTGGAACGGCAGC-3' (SEQ.ID.NO.:1)

and a 3' primer having the following sequence:

5'-AGAACCACCACCAGCAGGACGCGGACGGTCTGCCGGTGG-3'

(SEQ.ID.NO.:2).

The second PCR fragment was amplified with a 5' primer having the following sequence:

5'-GTCCGCGTCCTGCTGGTGGTGGTTCTGGCATTTATAATT-3 (SEQ.ID.NO.:3)

and a 3' primer that contained a BamHI site and having the following sequence:

5'-CCTGGATCCTTATCCCATCGTCTTCACGTTAGC-3' (SEQ.ID.NO.:4).

The two fragments were used as template to amplify GPR38, suing SEQ.ID.NO:1 and SEQ.ID.NO.:4 as primers (using the above-noted cycle conditions). The resulting 1.44 kb PCR fragment was digested with BamHI and cloned into Blunt-BamHI site of pCMV expression vector. Nucleic acid and amino acid sequences for human GPR38 were thereafter determined and verified.

Example 2
PREPARATION OF NON-ENDOGENOUS, CONSTITUTIVELY ACTIVATED GPR38

Preparation of the non-endogenous, constitutively activated human GPR38 receptor was accomplished by creating a V297 mutation (*see*, SEQ.ID.NO.:5 for nucleic acid sequence, and SEQ.ID.NO.:6 for amino acid sequence). Mutagenesis was performed using Transformer Site-Directed™ Mutagenesis Kit (Clontech) according to manufacturer's instructions. The two mutagenesis primers utilized, a lysine mutagenesis oligonucleotide (SEQ.ID.NO.:7) and selection marker oligonucleotide (SEQ.ID.NO.:8), had the following sequences:

5'-GGCCACCGGCAGACCAAACGCGTCCTGCTG -3' (SEQ.ID.NO.: 7) and

5'- CTCCTTCGGTCCTCCTATCGTTGTCAGAAGT -3' (SEQ.ID.NO.: 8), respectively.

Example 3
RECEPTOR EXPRESSION

Although a variety of cells are available to the art for the expression of proteins, it is most preferred that mammalian cells be utilized. The primary reason for this is predicated upon practicalities, *i.e.*, utilization of, *e.g.*, yeast cells for the expression of a GPCR, while possible, introduces into the protocol a non-mammalian cell which may not (indeed, in the case of yeast, does not) include the receptor-coupling, genetic-mechanism

and secretary pathways that have evolved for mammalian systems – thus, results obtained in non-mammalian cells, while of potential use, are not as preferred as that obtained from mammalian cells. Of the mammalian cells, COS-7, 293 and 293T cells are particularly preferred, although the specific mammalian cell utilized can be predicated upon the particular needs of the artisan.

On day one, $1X10^7$ 293T cells per 150mm plate were plated out. On day two, two reaction tubes were prepared (the proportions to follow for each tube are per plate): tube A was prepared by mixing 20μg DNA (*e.g.*, pCMV vector; pCMV vector with receptor cDNA, etc.) in 1.2ml serum free DMEM (Irvine Scientific, Irvine, CA); tube B was prepared by mixing 120μl lipofectamine (Gibco BRL) in 1.2ml serum free DMEM. Tubes A and B were then admixed by inversions (several times), followed by incubation at room temperature for 30-45min. The admixture is referred to as the "transfection mixture". Plated 293T cells were washed with 1XPBS, followed by addition of 10ml serum free DMEM. 2.4ml of the transfection mixture was then added to the cells, followed by incubation for 4hrs at 37°C/5% CO_2. The transfection mixture was then removed by aspiration, followed by the addition of 25ml of DMEM/10% Fetal Bovine Serum. Cells were then incubated at 37°C/5% CO_2. After 72hr incubation, cells were then harvested and utilized for analysis.

Example 4
REPORTER-BASED ASSAY: CRE-LUC REPORTER ASSAY

A method to detect Gs stimulation depends on the known property of the transcription factor CREB, which is activated in a cAMP-dependent manner. A PathDetect pCRE-Luc trans-Reporting System (Stratagene, Catalogue # 219075) was utilized to assay for Gs coupled activity in 293T cells. Cells were transfected with the

plasmids components of this system and the indicated expression plasmid encoding endogenous or non-endogenous receptor using a Mammalian Transfection Kit (Stratagene, Catalogue #200285) according to the manufacturer's instructions. Briefly, 400 ng pCRE-Luc, 80 ng pCMV (comprising the receptor) and 20 ng CMV-SEAP (secreted alkaline phosphatase expression plasmid; alkaline phosphatase activity is measured in the media of transfected cells to control for variations in transfection efficiency between samples) were combined in a calcium phosphate precipitate as per the manufacturer's instructions. Half of the precipitate was equally distributed over 3 wells in a 96-well plate, kept on the cells overnight, and replaced with fresh medium the following day. Forty-eight (48) hr after the start of the transfection, cells were treated and assayed for luciferase activity using a Luclite™ Kit (Packard, Cat. # 6016911) and "Trilux 1450 Microbeta" liquid scintillation and luminescence counter (Wallac) as per the manufacturer's instructions. The data was analyzed using GraphPad Prism™ 2.0a (GraphPad Software Inc.). Results are summarized in Figure 1.

Figure 1 represents an 83.1% increase in activity of the non-endogenous, constitutively active version of human GPR38 (V297K) (11,505 relative light units) compared with that of the endogenous GPR38 (1950 relative light units).

References cited throughout this patent document, unless otherwise indicated, are incorporated herein by reference. Modifications and extension of the disclosed inventions that are within the purview of the skilled artisan are encompassed within the above disclosure and the claims that follow.

Although a variety of expression vectors are available to those in the art, for purposes of utilization for both the endogenous and non-endogenous human GPR38, it is

most preferred that the vector utilized be pCMV. This vector has been deposited with the American Type Culture Collection (ATCC) on October 13, 1998 (10801 University Blvd., Manassas, VA 20110-2209 USA) under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purpose of patent Procedure. The vector was tested by the ATCC and determined to be viable. The ATCC has assigned the following deposit number to pCMV: ATCC #203351.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Behan, Dominic P.
Chalmers, Derek T.
Liaw, Chen W.
Lin, I-Lin

(ii) TITLE OF INVENTION: Non-Endogenous, Constitutively Activated Human G Protein-Coupled Orphan Receptor:GPR38

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Arena Pharmaceuticals, Inc.
(B) STREET: 6166 Nancy Ridge Drive
(C) CITY: San Diego
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 92122

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Burgoon, Richard P.
(B) REGISTRATION NUMBER: 34,787

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (619)453-7200
(B) TELEFAX: (619)453-7210

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ACCATGGGCA GCCCCTGGAA CGGCAGC 27

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AGAACCACCA CCAGCAGGAC GCGGACGGTC TGCCGGTGG 39

(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTCCGCGTCC TGCTGGTGGT GGTTCTGGCA TTTATAATT 39

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCTGGATCCT TATCCCATCG TCTTCACGTT AGC 33

(6) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2040 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

ACCATGGGCA GCCCCTGGAA CGGCAGC 27

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AGAACCACCA CCAGCAGGAC GCGGACGGTC TGCCGGTGGC C 41

(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTCCGCGTCC TGCTGGTGGT GGTTCTGGCA TTTATAATT 39

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCTGGATCCT TATCCCATCG TCTTCACGTT AGC 33

(6) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2040 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGGGCAGCC CCTGGAACGG CAGCGACGGC CCCGAGGGGG CGCGGGAGCC GCCGTGGCCC 60

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GTGACCGCTG TGTGCCTGTG CCTGTTCGTC GTCGGGGTGA GCGGCAACGT GGTGACCGTG 180

ATGCTGATCG GGCGCTACCG GGACATGCGG ACCACCACCA ACTTGTACCT GGGCAGCATG 240

GCCGTGTCCG ACCTACTCAT CCTGCTCGGG CTGCCGTTCG ACCTGTACCG CCTCTGGCGC 300

TCGCGGCCCT GGGTGTTCGG GCCGCTGCTC TGCCGCCTGT CCCTCTACGT GGGCGAGGGC 360

TGCACCTACG CCACGCTGCT GCACATGACC GCGCTCAGCG TCGAGCGCTA CCTGGCCATC 420

TGCCGCCCGC TCCGCGCCCG CGTCTTGGTC ACCCGGCGCC GCGTCCGCGC GCTCATCGCT 480

GTGCTCTGGG CCGTGGCGCT GCTCTCTGCC GGTCCCTTCT TGTTCCTGGT GGGCGTCGAG 540

CAGGACCCCG GCATCTCCGT AGTCCCGGGC CTCAATGGCA CCGCGCGGAT CGCCTCCTCG 600

CCTCTCGCCT CGTCGCCGCC TCTCTGGCTC TCGCGGGCGC CACCGCCGTC CCCGCCGTCG 660

GGGCCCGAGA CCGCGGAGGC CGCGGCGCTG TTCAGCCGCG AATGCCGGCC GAGCCCCGCG 720

CAGCTGGGCG CGCTGCGTGT CATGCTGTGG GTCACCACCG CCTACTTCTT CCTGCCCTTT 780

CTGTGCCTCA GCATCCTCTA CGGGCTCATC GGGCGGGAGC TGTGGAGCAG CCGGCGGCCG 840

CTGCGAGGCC CGGCCGCCTC GGGGCGGGAG AGAGGCCACC GGCAGACCAA ACGCGTCCTG 900

CGTAAGTGGA GCCGCCGTGG TTCCAAAGAC GCCTGCCTGC AGTCCGCCCC GCCGGGGACC 960

GCGCAAACGC TGGGTCCCCT TCCCCTGCTC GCCCAGCTCT GGGCGCCGCT TCCAGCTCCC 1020

TTTCCTATTT CGATTCCAGC CTCCACCCGC CGGTACTTCC CATCCCCCGA GAAAACCATG 1080

TCCTGTCCCC CAGGAGCTCT GGGGGACCCC AGGGCGCTTT GAGGGTGGGA TCCCCGGATC 1140

CGATTCAGTA ACCAGCAGTG CTTTTCCAGA GCCTCTGAGA CCAGAAAGGA GAGTTGGTAA 1200

TTCTTAATCC AACCACCTGT TAGATGCCAC AAATGAGGAG TCCTCACAGT GCTCTTGAGA 1260

AGACGAGGGA GATTTCATTA AGCTAAAATT TTTTATTTAA TGTTAAGTGA TGCTGAAGGC 1320

TAAAGTAAAC CTTGCTCGTA TCAAAAAGTA AAGATTGTGC AGACCTGTTG TAGAATTCTT 1380

TTCAACAGAG AACAGAAAAC TTGTCTCCGA AGTGGGTTTG TGGAAGGAAG CCTGCCAAGG 1440

CGGCTTGTTC AGAGAAATTG CTCCTTCTGG TTTATGTCCA GCCTTGATAA CACATATGGG 1500

AGCCTACTAT GCAGTTTTAA AGCAAGTATC CATGCAGCCT GCAGCCTGGT CATTTTTTCT 1560

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GCAGATGGTT CCTTGTCGGG GTGGGGGGTT TATTTGCTTC CCAATGCTTT TGTTAATCCC 1680

GGTGCTGTGT CTTATGTTGC AGTGGTGGTG GTTCTGGCAT TTATAATTTG CTGGTTGCCC 1740

TTCCACGTTG GCAGAATCAT TTACATAAAC ACGGAAGATT CGCGGATGAT GTACTTCTCT 1800

CAGTACTTTA ACATCGTCGC TCTGCAACTT TTCTATCTGA GCGCATCTAT CAACCCAATC 1860

CTCTACAACC TCATTTCAAA GAAGTACAGA GCGGCGGCCT TTAAACTGCT GCTCGCAAGG 1920

AAGTCCAGGC CGAGAGGCTT CCACAGAAGC AGGGACACTG CGGGGGAAGT TGCAGGGGAC 1980

ACTGGAGGAG ACACGGTGGG CTACACCGAG ACAAGCGCTA ACGTGAAGAC GATGGGATAA 2040

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 680 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

MGSPWNGSDGPEGAREPPWPALPPCDERRCSPFPLGALVPVTAV

CLCLFVVGVSGNVVTVMLIGRYRDMRTTTNLYLGSMAVSDLLILLGLPFDLYRLWRSR

PWVFGPLLCRLSLYVGEGCTYATLLHMTALSVERYLAICRPLRARVLVTRRRVRALIA

VLWAVALLSAGPFLFLVGVEQDPGISVVPGLNGTARIASSPLASSPPLWLSRAPPPSP

PSGPETAEAAALFSRECRPSPAQLGALRVMLWVTTAYFFLPFLCLSILYGLIGRELWS

SRRPLRGPAASGRERGHRQTKRVLLVVVLAFIICWLPFHVGRIIYINTEDSRMMYFSQ

YFNIVALQLFYLSASINPILYNLISKKYRAAAFKLLLARKSRPRGFHRSRDTAGEVAG

DTGGDTVGYTETSANVKTMG

(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGCCACCGGC AGACCAAACG CGTCCTGCTG 30

(9) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

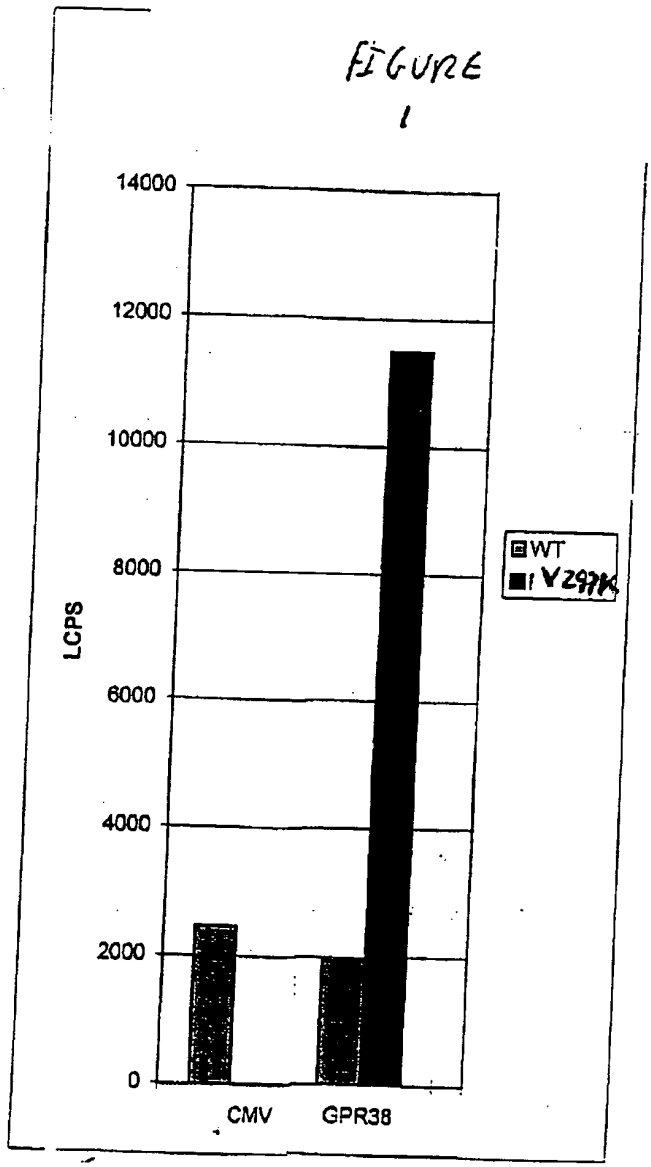
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CTCCTTCGGT CCTCCTATCG TTGTCAGAAG T 31

ABSTRACT

The invention disclosed in this patent document relates to transmembrane receptors, more particularly to G protein-coupled receptors for which the endogenous ligand is unknown ("orphan GPCR receptors"), and most particularly to a mutated (non-endogenous) version of human GPR38, with such mutated version being constitutively active.

FIGURE 1


14000
12000
10000
8000
6000
4000
2000
0
LCPS
CMV
GPR38
WT
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